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Introduction: Meningioma is the most common primary brain tumour and is classified as benign (WHO I, ~80%), a-typical (WHO II, ~15-20%) and anaplastic/malignant (WHO III, ~1-3%). The 3-year recurrence rate in WHO I meningioma is about 50% and it is much greater in WHO II and III.
Recent studies showed that cyclin D1 and E1 positively correlated with meningioma grade and higher rates of recurrence, suggesting these proteins as potential prognostic markers for meningioma.
Here, we show that overexpression of cyclin D1-D2 and E1 in malignant meningioma is driven by downregulation of the miR-15 family members (miR-15a-15b-16-195-497) via GATA4, a transcription factor overexpressed in WHO III meningioma cells and tissues. Therefore, we propose GATA4 as a novel possible biomarker for monitoring meningioma progression.
Material and Methods: Meningioma (MN) specimens were collected from consented patients according to the ethical approval for this study. All cell lines and primary MN cells were isolated from tumour specimens and cultured following recommended conditions.
Real Time PCR was conducted using TaqMan® reagents (Applied Biosystems), according to the manufacturer's instructions following the $2^{-\Delta\Delta C_T}$ method. *In silico* studies were conducted using TargetScanHuman7.1 to search for putative miRNA targets.
Probability (p) values were calculated using the Student's t-Test or the ANOVA one-way analysis of variance, led by the GraphPad Prism 5.01 and MS Excel 2016 software (p values $<0.05 \pm$ SEM).
Results and discussion: Proteomic analysis showed an increase of cyclin D1 in Ben Men-1, primary WHO I and KT21-MG1 cells, and an increase of cyclin D2 in KT21-MG1 cells only. *In silico* studies highlighted that cyclin D1-D2-D3 and E1 are targeted by the miR-15 family members. QPCR showed that miR-195 and -497 are downregulated in WHO II and III samples when compared to WHO I (3.38 and 6.02 folds, respectively; p=0.02); these results were consistent in cell culture exosomes.
Analysis of GATA4 revealed that the protein is highly overexpressed in KT21-MG1 cells compared with both Ben Men-1 and primary meningioma cells (17940.19 and 4899.34 folds, respectively, p=0.03). These results were consistent in tissues (2640.53 folds).
Conclusion: Our data show that all members of the miR-15 family are downregulated in WHO III meningioma cells and tissues, suggesting that they may

contribute to control tumour progression, characterised by higher expression of cyclin D1-D2 and E1. In addition, WHO III cells and tissues are characterised by a considerable overexpression of the transcription factor GATA4, which is involved in miR-15 family regulation. Ongoing studies will address whether these findings may represent potential diagnostic and/or prognostic biomarkers by analysing circulating exosomes, to improve the diagnosis and stratification of meningiomas.
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